1. INTRODUCTION

Parkinson's disease (PD) is a pathological condition that has been known for centuries through early Greek scientific descriptions, traditional Indian texts and ancient Chinese sources. However, it was first medically described as a neurological syndrome in 1817 when James Parkinson published a detailed medical essay on the shaking palsy. The most complete pathologic analysis of Parkinson's disease and the clear description of the brain lesions were performed in 1953 by Greenfield and Bosanquet when low dopamine presence were identified in the brains of PD patients (Parkinson, 2002).

Today, the PD movement disorder is the most frequent neurodegenerative condition of the aging brain just behind Alzheimer's disease. According to the 2005 report from the World Health Organization, the number of PD cases worldwide was expected to rise dramatically due to the increase of life expectancy. In the 1960s, the drug Levodopa was first introduced to treat the symptoms and has since become the "gold standard" in medication. Since then, research on PD has been focused on finding efficient symptomatic treatments using patient-based research. Discovery of 6-Hydroxydopamine and 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the neurotoxins which causes permanent PD symptoms, led to intense research and discoveries on the mechanisms responsible for dopaminergic neuron vulnerability and allowed to experiment with neuroprotective strategies (Langston et al., 1983).

As we enter the new century, Parkinson's disease ranks among the most common late life neurodegenerative diseases, affecting approximately 1.5% to 2.0% of the population over the age of 60. The causes of Parkinson’s disease (PD), the second most common neurodegenerative disorder, are still largely unknown. Current thinking is that major gene mutations occur in a small proportion of all cases and in most cases; non-genetic factors play a part, probably in interaction with susceptibility genes. Numerous epidemiological studies have been carried out to identify such non-genetic risk factors, but most were small and methodologically limited. (Lonneke and Monique., 2006)

PD can occur at any age, but is mainly a condition of middle and later life, about 1% of the over-65s and 2% over-80s age groups, more common in white people in Europe and North America.
Lower rates are seen in China, Nigeria and Sardinia. The disorder occurs in all races but is somewhat more prevalent among Caucasians. The incidence varies little between sexes. Life time risk of PD is 1 in 40 (Talene et al., 2009).

The substantia nigra, part of the extrapyramidal system, is the source of dopaminergic neurons that terminate in the striatum. Each dopaminergic neuron makes thousands of synaptic contacts within the striatum and therefore modulates the activity of a large number of cells. These dopaminergic projections from the substantia nigra fire tonically, rather than in response to specific muscular movements or sensory input. The striatum is connected to the substantia nigra by neurons that secrete the inhibitory transmitter GABA at their termini in the substantia nigra. In turn, cells of the substantia nigra send neurons back to the striatum, secreting the inhibitory transmitter dopamine at their termini. This mutual inhibitory pathway normally maintains a degree of inhibition of the two separate areas (Fearnley and Lees., 1991). Nerve fibers from the cerebral cortex and thalamus secrete ACh in the neostriatum, causing excitatory effects that initiate and regulate gross intentional movements of the body. In Parkinson’s disease, destruction of cells in the substantia nigra results in the degeneration of neurons responsible for secreting dopamine in the neostriatum. Thus the normal modulating inhibitory influence of dopamine on the neostriatum is significantly diminished, resulting in the parkinsonian degeneration of the control of muscle movement (Vingerhoets et al., 1994).

The primary pathological characteristics of PD are the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and reductions in their termini within the dorsal striatum. This lead to profound and irreversible striatal dopamine loss. Many factors are speculated to operate in the mechanism of cell death of the nigrostriatal dopaminergic neurons in PD, including oxidative stress and cytotoxicity of reactive oxygen spices (ROS), disturbances of intracellular calcium homeostasis, exogenous and endogenous toxins, and mitochondrial dysfunction (William., 2003). Moreover, increased risk of localized oxidative damage for dopaminergic neurons is linked to dopamine metabolism itself. With the exception of rare familial forms, the majority of PD cases are sporadic and also due to mitochondrial defects at complex I. Moreover, the presence of ubiquitinated and misfolded proteins suggests the dysregulation of
protein assembly or defects in protein degradation pathway as a critical part of disease pathogenesis. Misfolding and abnormal degradation of brain proteins are linked to dopaminergic neuronal death (Orth and Schapira., 2002).

The most effective Parkinson's drug is levodopa, which when passes into the brain is converted to Dopamine (DA). Levodopa is combined with carbidopa to create the combination drug Sinemet. The carbidopa protects from premature conversion to DA outside the brain, hence prevents nausea.

**In prolonged levodopa therapy, the apparent buffering capacity is lost and the patient’s motor state** may fluctuate dramatically with each dose of the drug. A common problem is the development of wearing off phenomenon and each dose of levodopa effectively improves mobility for a period of time, about 1 or 2 hours, but rigidity and akinesia return rapidly at the end of dosing interval. Increasing the dose and frequency of administration can improve this situation, but this often is limited by the development of dyskinesias, excessive and abnormal involuntary movements. Dyskinesias are observed most often when the plasma levodopa concentration is high although in some individual’s dyskinesia or dystonia may be triggered when the level is altered. These movements can be as uncomfortable and disabling as the rigidity and akinesia of PD. In the later stages of PD, patients may fluctuate rapidly between being "off", having no beneficial effects from their medications and being "on" but with disabling dyskinesias, a situation called on/off phenomenon (Neha et al., 2007).

Unlike many other neurodegenerative diseases, there is effective symptomatic therapy for Parkinson's disease that can provide most patients with several years of satisfactory quality of life and response to treatment (Lepoutre et al., 2007). The key limiting points in PD therapy include:

- No therapy has yet been shown to slow or reverse the disease, although clinical trials of several candidates have shown intriguing results.
- Levodopa continues to be the most effective treatment for motor symptoms, and all patients eventually require it.
• Long-term complications of dopaminergic therapy are a concern that drives decision-making early in the treatment program.
• Non-motor symptoms, especially depression and anxiety, are important targets of therapy.
• Surgical treatment has become a mainstay of late-stage management, although not all patients can afford it or are appropriate candidates.
• Cell transplant therapies are still experimental.
• Non-pharmacological treatments remain an important part of a comprehensive treatment program.

The mechanisms responsible for the preferential loss of DA neurons in PD have been debated for decades. A widely held theory implicates DA itself, suggesting that oxidation of cytosolic DA (and its metabolites) leads to the production of cytotoxic free radicals (Greenamyre et al., 2004). However, there are reasons to doubt that type of cellular stress alone is responsible for the loss of DA neurons in PD. For instance, there is considerable regional variability in the vulnerability of DA neurons in PD, with some areas being devoid of pathological markers (Matzuk and Saper, 1985; Damier et al., 1992).

Moreover, L-DOPA administration (which relieves symptoms by elevating DA levels in PD patients) does not appear to accelerate disease progression (Fahn, 2005), suggesting that DA itself is not a significant source of reactive oxidative stress, at least in the short term. It is recently reported that Ca\(^2+\) entry through L-type channels stimulates DA metabolism in SNc DA neurons, pushing cytosolic DA concentrations into a toxic range with L-DOPA loading (Mosharov et al., 2009). As noted later in the text, the oxidant stress created by DA could interact with other factors to promote cell loss. However, the frank death or phenotypic decline of a variety of non DA neurons in PD argues that DA itself is not likely to be the principal culprit in the disease.

In a recent epidemiological study on the potential effect of the use of Ca\(^2+\) channel blockers on the evolution of Parkinson’s disease, about two-thirds of the patients were treated with Nimodipine. No relation between the use of such drugs and the incidence of Parkinson’s disease was found. However, neither Nimodipine nor Amlodipine are potent blockers of the Cav1.3 channels that underlie pacemaking in SNc dopamine neurons. Other nominal Ca\(^2+\) channel blockers Flunarizine and Cinnarizine worsen the symptoms of Parkinson’s, but this effect is attributable to their antagonism of dopamine receptors, not their block of Ca\(^2+\) channels (Jan and Parys., 2011).
contrast, retrospective examination of patients treated for hypertension with Dihydropyridine the class of drug used to induce reversion of the SNC dopamine neuron pacemaking and neuroprotection in mice revealed a lower than expected incidence of Parkinson’s disease. In moving forward, there are several issues that need to be considered, one of the important factor is the choice of drug. In the absence of a selective Cav1.3 Ca\(^{2+}\) channel antagonist, Dihydropyridine such as Nitrendipine offer the best therapeutic options. Dihydropyridines are more selective blockers of L-type channels than are other Ca\(^{2+}\) channel blockers approved for human use, and have good brain bioavailability. However, most members of this drug class, including nimodipine and Nitrendipine, are more potent blockers of Cav1.2 than of Cav1.3 channels (James and Surmeier., 2010).

Calcium is a critical cation both physiologically and pathologically. Its control is of fundamental significance to the life and death of the cell. Calcium plays a key physiological requirement for both sperm motility and the fertilization response, and calcium is a pathological contributor to the cell-death process. Thus in a very real sense we are conceived in a moment of calcium mediated enthusiasm, we die from an excess of calcium and we are laid to the rest under tombstone of calcium carbonate. Voltage gated calcium channels are important contributors to both the life and death of the cell. Under tightly controlled conditions calcium is a critical cellular messenger mediated multiple stimuli –response coupling events when uncontrolled, calcium is a pathological signal mediating cell death and destruction. The control of cell calcium is thus a substantial preoccupation of the cell, which describes the relation between calcium, membrane integrity and cellular permeability (Gorgy et al., 2006).

Given the ubiquity of calcium as a messenger and the widespread distribution of the voltage gated calcium channels in virtually all cell and organs, the critical issue of selectivity of drug action arises. The available calcium antagonists owe their pharmacological and therapeutic profile to their ability to interact with considerable selectivity at one of the calcium oblilized processes the L-Type or Ca\(_{1,2}\), channel subclass. The widespread distribution of voltage gated calcium channels in virtually all cell and organ types in cardiovascular systems, non vascular smooth muscle, secretory, and neuronal systems indicates that it is appropriate to consider a multiplicity of actions of appropriate calcium channel antagonist for various disorders (Münchau., 2000).
In a recent work it has shown that controlled opening of L-type Ca\(^{2+}\) channels in SNc DA neurons creates a basal mitochondrial oxidant stress (Guzman et al., 2010). A two-photon laser scanning microscopy was used to monitor mito-roGFP in brain slices from young adult mice, it was found that the engagement of plasma membrane Cav1.3 L-type calcium channels during normal autonomous pacemaking created an oxidant stress in the mitochondria that was specific to the vulnerable SNc DA neurons and not apparent in neighboring VTA DA neurons. This study induces us to take a lead molecule to specifically target Cav1.3 L-type calcium channels for reducing SNc DA neuronal injury.

Neuroinflammation is one of the other prominent factor in the development of PD. Microglia is a bone marrow derived macrophage-lineage cells that enter the brain early during embryogenesis and develop in parallel with the maturation of the nervous system. They are the resident phagocytes of the CNS and can react promptly in response to brain insults of various natures, ranging from pathogens to aggregated proteins and to more subtle alterations in their micro-environment such as alterations in ion homeostasis that can affect pathological processes (Mosley et al., 2006). In the normal brain, microglial cells are in a resting state, their cell bodies barely visible. However, in pathological settings, resting microglial cells quickly proliferate, become hypertrophic, and express a large number of marker molecules such as the macrophage antigen complex-I (MAC-1), and a variety of proinflammatory cytokines such as interferon-γ (IFN-γ), interleukin 1-β and upregulate enzymes such as inducible nitric oxide (iNOS) and cyclooxygenase (COX) 1 and 2. The brain area that encompasses the substantia nigra has the highest density of microglia in the brain. There appears to be a graded response with differential expression depending on the severity of the insult. A consequence of activation of microglia is the production of reactive oxygen species. They can originate from NADPH oxidase, which produces superoxide (\(O_2^-\)); COX-2, which produces free radicals as a byproduct of prostaglandin synthesis; and from inducible nitric oxide synthetase (iNOS), which generates NO. The reaction of O\(_2\) with NO generates peroxynitrile ONOO\(^{-}\), which is strongly implicated in PD pathogenesis. Hence the enzyme COX as well as inflammatory mediators such as nitric oxide increases in PD (Beal., 2003). In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced animal model of PD, mechanisms mediating inflammatory reactions are reported to contribute to the neuronal damage. Supporting these findings, the non selective COX-inhibitor, aspirin has been reported to confer neuroprotection in
MPTP-induced dopamine (DA) depletion in mice. However, other COX activity inhibitors like paracetamol, indomethacin, diclofenac or COX expression inhibitor dexamethasone were found to be ineffective in protecting neurons against MPTP neurotoxicity (Sairam et al., 2003).

Taken together these information, it is suggested that the microenvironment of the parkinsonian SN is modified during the pathological process. Such changes not only involve neurons and glial cells but also peripheral immune cells and even brain capillaries. Indeed, there is changes in neuronal vascular relationships in the SN in PD (Issidorides et al., 1971)

More recently, a report says a 2.5-fold increase in patients with Parkinson’s disease in the number of nuclei of blood vessel endothelial cells in the SN where DA neurons degenerate (Faucheux et al., 1999).

This increase was selective for the affected brain regions since it was only very slight in the DA brain regions less affected by the pathological process. Such a modification of the vascular microenvironment of DA neurons may alter the accumulation drawing and transport of nutrients or toxic compounds in PD. Among the possible consequences, changes in vascularisation density could contribute to iron accumulation, since an increase in lactotransferrin and lactotransferrin receptor expression has been observed on blood vessels in the SN of patients with PD (Leveugle et al., 1996; Faucheux et al 1995).

When considered together, these data suggest a potential role of glial cells and inflammatory processes in the pathophysiology of PD. Moreover, strong support for this hypothesis came from the study of young drug addicts who developed a parkinsonian syndrome after 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) intoxication (Langston et al., 1983). Langston et al., 1983 reported a post-mortem neuropathological study of three subjects with MPTP-induced parkinsonism. Interestingly, gliosis and clustering of microglial cells around nerve cells were detected despite survival times ranging from 3 to 16 years. These findings not only indicated ongoing nerve cell loss after a time-limited insult, but also suggest that activated microglial cells may perpetuate neuronal degeneration. One may thus speculate that after a primary insult of environmental or genetic origin, the glial reaction may maintain the degeneration of DA neurons through progressive inflammation (Chen et al., 2003).
A strong glial reaction and inflammatory cytokine release have also been described in various animal models of PD, such as 6-hydroxydopamine (6-OHDA) brain injection, MPTP, rotenone or annonacine peripheral injection. One of the oldest techniques used to induce parkinsonism in animals is to inject 6-OHDA into the brain parenchyma, leading to the degeneration of the nigrostriatal DA pathway. Microglial activation has been described in this model after both medial forebrain bundle or striatal injection of the toxin (Akiyama and McGeer., 1989; He et al., 2001; Depino et al., 2003). In a recently tested hypothesis, the activation of peroxisome proliferator-activated receptor-g (PPAR-γ), a member of the nuclear receptor super-family has been shown to inhibit inflammatory processes, probably by counteracting NFkB activation and inhibiting the c-jun kinase pathways (Combs et al., 2000). The anti-inflammatory drugs such as minocycline and propiphenazone have also been shown to protect DA neurons against MPTP intoxication. Interestingly, these compounds have also been shown to reduce the formation of nitrotyrosine produced during MPTP intoxication by iNOS (Wu et al., 2002). And also the neuroprotective effects of minocycline in animal models of PD have been evaluated, as some groups of investigators have even reported a detrimental positive effect of this compound (Diguet et al., 2004). Similarly, inhibitors of cyclooxygenase-2, the rate-limiting enzyme in prostaglandin-E2 synthesis, have also produced controversial results. Yet, such variable results are explained by the pharmacological profile and the specificity of the drugs used (salicylate, aspirin, meloxicam, indometacin, paracetamol, diclofenac, ibuprofen, etc.), since COX-2 knockout animals were relatively preserved against MPTP intoxication (Hirsch et al., 2003; Teismann et al., 2003).

It is probable that neuroinflammatory processes may participate in the cascade of events leading to neuronal degeneration in PD. Yet, these events are not specific to this neurodegenerative disease as they have also been described in other neurological disorders such as Alzheimer’s disease, multiple sclerosis, AIDS dementia, trauma and stroke. The inhibition of these inflammation processes in animal models of the disease prevents neuronal degeneration, it is likely that they are instrumental in preventing cell demise. The major challenge in our study is to make out the possible neuro protection offered by Aspirin and indomethacin in rat models. The basic and advanced pharmacological techniques will be executed to rule out the reduction in neuronal degeneration in parkinson’s model.